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THE AËRATION OF ORGANS AND TISSUES IN MIKANIA AND OTHER PHANEROGAMS.*

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Introduction.

The object of this thesis is to extend the knowledge relating to the respiration of plants. The adaptation of organs and tissues to facilitate respiration is, I believe, much more frequent than has generally been recognized. The investigations were rendered particularly interesting and valuable through the discovery of the peculiar root system of *Mikania scandens* and certain other closely related species. Comparatively little has been done by American and English botanists upon this subject. Foreign investigators, especially the French, have done much more along this line. Professor Wilson's work upon "cypress-knees" (37 and 38)† has been important. Schrenk (36) has studied the aërating tissues of Nesæa, but was non-committal as to their function.

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†The numbers in the text correspond to the number of the paper in "literature" at the end of the paper.

Direct analyses of the gases contained in the intercellular spaces have been made repeatedly with the uniform result of finding in non-chlorophyll-bearing organs, and in chlorophyll-bearing organs when kept in the dark, a diminution of oxygen and an increase of carbon dioxide as compared with the external air.

The bibliography includes the more important investigations upon (1) respiration in general, (2) structural peculiarities, such as may facilitate respiration. Plants showing these modifications are widely separated genetically; nevertheless the modifications are along certain clearly defined lines of development.

Respiration a Universal Attribute of Organisms.

The two external elements most essential to life are *air* and *water.* The structural adaptation of organs to secure aëration of tissues in the higher animals are elaborate and usually highly complicated. The organs of respiration become more and more simple as one descends the scale of animal life, until forms are found the existence of which depends upon their bodies being bathed with aërated water. Simple plants depend likewise upon the air held in solution in the water for aëration of their tissues. They absorb from the air in the water sufficient oxygen to enable them to live. The adaptation of organisms to environment is a subject which has always interested naturalists. Claude Bernard has said, "tous les etres vivants sont aquatique." It is equally true that all beings must, in order to live, have air. One might legitimately and reasonably ask, Why do not plants of great bulk smother their own tissues? Why do not stems which grow in the water, and are thereby limited as to the amount of gases surrounding them, modify their structure when they emerge and grow in air? Or, to put the same idea in a general way, Ought we not to expect a change in the structure of a stem when its environment is changed? These questions find an answer when we investigate plant structure grown under different conditions.

If plants are green they may throw off as a waste product enough oxygen to supply their own immediate needs, or indeed much more than that, but all plants and all plant-parts not possessing chlorophyll must have oxygen from some source to sustain life. It has long been taught that animals use up oxygen in their respiration and plants give it off. By their mutual action the air is kept normally charged with oxygen. This doctrine has led to much confusion.

The Setting Free of Oxygen by the Plant.

It should be distinctly understood that all organisms, both plant and animal, respire alike. They fix oxygen and give off carbon dioxide. This process oes on under all circumstances in living tissues—in dormant organs slowly, in rapidly growing ones very fast. Green plants have a power connected with their process of nutrition which no other organisms have. This is the fixation of carbon and the setting free of oxygen. The production of oxygen, then, is incidental to their method of nutrition and may properly be considered as a waste product of assimilation. This oxygen may be of importance to the plant, but it is not essential. Plants will grow without assimilation provided they have sufficient food stored to enable them to build up their tissues. Some tubers when grown in the dark will grow up into tolerably perfect plants and produce flowers without assimilating at all. This experiment may be easily conducted with a hyacinth. If the tuber of this plant be grown in the dark, a vegetative growth nearly normal is made, and such plants will often produce well-developed and highly colored flowers.

The Necessity for Modification of Tissues and Organs to Facilitate Respiration.

If respiration be one of the primary functions of organisms, it is to be expected that modifications to increase the facility and certainty of its accomplishment will be found among the more highly organized plants. The modification of organs to secure the best conditions for aëration is the direct result of an evolution of forms best adapted to live. Indeed, one might justly say that respiration is the first if not the most important of the functions which are carried on. The first signs of germination in a seed is the enlargement of spaces between the cells. In some cases the intercellular spaces become comparatively large before active cell division begins. During the succeeding life of the plant, where the massiveness of tissue or lack of aëration in the surrounding medium makes it desirable, provision is made for aëration either by intercellular spaces without any regular organization of tissues, or (2) by a special tissue organized to increase the ease with which air is supplied and regulated.

The Special Part of the Plant to which Air Must be Brought.

The part of the plant which is to be aërated is that which is alive and active. Much of the tissue making up a plant is not alive and active. It is the active protoplasm of living cells that is the seat of life in the plant, and it is that substance which must be both aërated and supplied with water. The protoplasm of a mature active cell usually assumes a definite form and arrangement of parts within the cell-wall. The protoplasm constantly changes its position as well as its chemical and physical properties, but nevertheless certain parts may be distinguished. The cell-wall is lined with a delicate membrane of protoplasm known as the ectoplasm. This lies in very close contact with the wall in the living cell, but contracts from it when the tissue is placed in certain reagents. Sometimes the ectoplasm of two adjacent cells are visibly connected. This continuity of protoplasm occurs in the cortical tissues of many woody plants. It has been asserted that the protoplasm of all living adjacent cells is continuous, being connected by invisible threads of protoplasm. Some have gone so far as to say that this can be demonstrated. The irritability of plants and the powers which plants have of absorbing and re-forming their walls can perhaps best be explained upon this ground.

The Permeability of the Cell-wall.

It is to the ectoplasm and the nucleus that whatever the cell needs to live upon must come. It was once believed that cutinized cell-walls were impervious to gases. This view is now entirely abandoned. It was Graham, an English physicist and chemist, who first formulated the laws upon which the modern idea of the dialyses of gases permeating a rubber membrane are based. Barthelemy hastened to apply these laws to plant membranes, such as the epidermis of the leaf or stem. He even satisfied himself that stomates and other openings in cutinized membranes were of minor importance to the plant in its exchange of gases. This was in direct opposition to Sachs' conclusions. The tendency of recent investigations is to establish an exchange of gases between the internal air chambers in the plant and the surrounding air, both by effusion through the stomates, lenticels, and other openings in the epidermis, and also by diffusion of gases through the cell-walls even when they are cutinized.

Historical.

With the founders of plant anatomy as a science began the first genuine knowledge of plant respiration. Grew's statements may be taken as representing the views of his time. In speaking of the motion and course of air, he says (1): "Although the air enters in part at the trunk and other parts, especially in some plants, yet its chief entrance is at the root. * * * The air being a springy body, it insinuates into all the holes and crannies of the earth and so is plentifully mixed therewith. Whereupon, as the sap enters the root, more or less air still intrudes itself together with it." Grew insists that the air and the sap of the plant bear a certain relation to each other.

In 1727 Hales made experimental researches upon plants. He asserts that air is essential to the production of growth in plants and animals in two ways: First, it "invigorates their juices while it is in an elastic state," and, second, "it contributes much to the firm union of the constituent parts when it has become fixed."

Priestley's discovery in 1779 marks an era in the science of plant physiology. The purification of the air by plants was one of the provisions which nature had made to offset the destruction of pure air by animals. Here began the confusion which covered the subject of plant respiration so long. It even is said today that animals breathe out impure air and plants breathe out (?) pure air.

Ingenhouss in the same year carried the subject a little farther and established the fact that plants purify air much more rapidly in sunshine than they do in shade. At this time oxygen, the properties of which had come to be quite well known, was called "vital air."

At the beginning of the present century De Saussure (2 and 3), an able French chemist, took up the researches of Priestley and Ingenhouss, and since that time the most important investigations upon the subject have been made in France and Germany. De Saussure distinguished clearly between respiration and assimilation, and further showed that oxygen respiration was necessary to plant life. He grew certain seedlings in closed vessels and analyzed the gases exhaled in a certain length of time. It was found that there was always the consumption of oxygen and the setting free of carbon dioxide. During the first stages of germination less oxygen was consumed than in the later stages. He also determined the composition of air in his native town with particular reference to the amount of carbon dioxide it contained. The results of these researches were given in the Ann. de Chim , 1830, xliv, p. 5.

Dutrochet (4) investigated the relation of air to the stomates and intercellular spaces and the vessels. He also set forth how gases might diffuse by osmosis through a membrane such as the epidermis of an organ. He, however, made the mistake of considering the waste oxygen from assimilation as the chief agent in respiration and the oxygen absorbed directly from the air as subsidiary to this. He perpetuated the use of the terms nocturnal and diurnal respiration of plants.

About 1860 the question of the relation of gases to plants assumed a new phase. Bonnet, in 1769, had noticed bubbles of some gas upon the leaves and stems of plants. It is doubtful whether these were disengaged from the plant or were only superficial bubbles. Not until 1849 did any one notice, or at least rightly interpret, the regular disengagement of bubbles from submerged plants exposed to light. The credit for this discovery belongs to Cloez and Gratiolet (7). They made analyses of the gas found in these bubbles and found that not only did they contain oxygen and carbon dioxide, but also nitrogen. They were thereby led into the error of supposing that nitrogen is one of the products of plant assimilation.

Boussingault (8 and 9), with the advantage of many years of experience in chemical investigations, took up the subject of the real origin of nitrogen. He also found, as had Cloez and Gratiolet, that there was always nitrogen in the bubbles of air which escape from submerged plants, but believed that it came from the water instead of the plant. This he succeeded in proving. Cloez and Gratiolet, in 1863, replied to M. Boussingault and maintained the theory that neither carbon dioxide nor hydrogen carbon are set free by the plant.

Van Tieghem (16) made close observations upon the way in which bubbles escape from the plant in presence of light. He noticed that bubbles continued to escape even after dark and concluded that the cause for this was the continuation of the influence of light.

Sachs' work upon experimental plant physiology appeared about this time. He emphasized the importance of stomates and their power of opening and closing. His principles are accepted by most

English and American students at the present time. From the first publication of his work above referred to, there have been investigators who were convinced that the stomates were of much less importance than Sachs believed them to be. The exchange of gases, according to their observations, was principally by diffusion through the epidermal membranes. Barthelemy (13 and 14) even went so far as to call the stomates "safety-valves" which permit the escape of nitrogen and watery vapor when the pressure of internal gases becomes great.

So much has been written by both sides upon the subject that it is difficult to separate what is demonstrated fact from mere opinion. Some have written as though the principles of plant physiology were to be based upon matters of opinion instead of experimental demonstration. From what has actually been proven, the following may be safely depended upon as a basis for our studies:

1. The gases of the atmosphere penetrate all the living tissues of plants.
2. This takes place either by diffusion through the epidermal coverings of all organs or by effusion through stomata, lenticels, and kindred natural or accidental epidermal openings.
3. The passage of gases into non-assimilating tissues is generally by diffusion. Air passes to the cells in which chlorophyll exists both by diffusion and effusion.
4. Air dissolved in water is practically identical in composition and pressure with the air above the surface of the water, providing the water be normally aërated. The tendency is for this air to be richer in oxygen than free air.
5. Diffusion of gases takes place with greater facility through the epidermal membranes of submerged plants than it does through the epidermal membranes of terrestrial plants.
6. Diffusion between the gases dissolved in water and the gaseous contents of an intercellular space takes place as readily as those dissolved in water would with the gases of the atmosphere.
7. All the living cells in the non-chlorophyll-bearing organs of submerged plants or submerged plant parts depend for their respiratory gases either upon the gases dissolved in the surrounding water or, in case a portion of the plant is above the water, upon the passage of gases through the intercellular spaces or other passages from the parts above water to the tissues below the surface.
8. The variations of pressure produced in the cell by chemical

transformations due to respiration or to chlorophyllous assimilation are at least thirty times more for oxygen than for carbon dioxide.

The physiological significance of intercellular spaces becomes very great when the above points are considered. Their constant presence and large size in aquatic plants at once point to their having some connection with aëration, and their presence in organs which show aërotropic tendencies must be admitted as the strongest evidence of their office.

So far I have attempted, from the literature as well as from generally accepted principles, to set forth the necessity for the aëration of submerged plants or submerged organs.

Deherain (11) has gone so far as to assign the death of the fish in a pond to the withdrawal of oxygen in the water by certain plants. He analyzed the water and found that it had considerable quantities of carbon dioxide in it but no oxygen. It does not appear in the report of this circumstance why the fish instead of the plants may not have been the active agents in using up the oxygen of the water. There can be no doubt, however, about the depletion of the oxygen of the water by non-chorophyll-bearing plants and plant parts. This is especially apt to be the case in plants that grow in stagnant pools and muddy places. It is highly probable that these plants will find themselves without the requisite amount of oxygen to carry on their functions and special adaptations will be brought about.

Classification of Aërating Structures.

The aëration of submerged organs may be brought about in two ways:

I. By the modification of an organ, usually a root, (1) either so as to expose greater surface to the surrounding medium or (2) by changing the direction of growth so as to bring the organ to the surface of the soil or water.

II. By modification of tissues (1) in such a way as to enable plants to conduct gases to the more deeply submerged parts or (2) merely using the intercellular spaces as "regulators of air." Usually these two modifications go together. Under I would be included "cypress-knees," the aërotropic roots of *Jussiæa*, *Mikania*, *Eupatorium*, etc., as well as many other plants not noticed in this connection. Under II will be included the plants which have the so-called aërenchyma tissue and many others which have the same modification of tissues not cortical. This would include such plants as *Menyanthes*, *Eleocharis quadrangulata*, and the water lilies. The tissue modified may be the primary tissues of the organ, as in rhizoma of *Menyanthes*, or it may be in the tissues formed by secondary thickenings, as in *Nesea* and the stems (not the roots) of *Jussiæa*.

Mikania scandens has both a modification of organs and a modification of the primary tissues of the root.

Mikania Scandens.

General Remarks.

This is an interesting species aside from the special feature which is under consideration. The genus *Mikania* is a large one. In Bentham & Hooker's "Genera Plantarum" 60 species are accredited to it. Most of the species are found in Central and South America. Only one species occurs in the northeast United States, viz., *Mikania scandens*. The genus has its center of distribution far to the south. To the northward the species become fewer, until in the states bordering upon the Gulf of Mexico there are only two or three species. Finally, as has already been said, but a single one of the sixty species is found in the northern Atlantic states. It might be well to add here that this is by no means the only genus of the order Compositæ found in the United States which has its center of distribution in the Southern Hemisphere.

The species of the closely related genus *Eupatorium* are distributed in nearly the same way. Certain species of this genus also have aërotropic roots. Other noteworthy cases of aërotropic roots are the cypress-knees and the *Jussiæs* of South America, upon which the first investigations upon aërating organs were made. If it should be that most of the cases of natural aërotropism should occur in South American plants or in plants having a South American origin, the fact would be an interesting one. In the meantime observations must be made upon plants related to both these groups in different localities, and there seems to me but little doubt but that there will be found many other species with the same peculiarities.

My conclusions in regard to the aëration of submerged plants or submerged parts of plants through the agency of their roots depend largely upon investigations upon the root system of *Mikania scandens*. These investigations extend over a period of three years. The plants studied were from the Oswego river, along the shores of which the plant grows in considerable abundance. The peculiarities in the root system of *Mikania* led me to look for similar structures in other species, and I have found them in a few cases.

The occurrence of similar structures have been recorded by European botanists. These were upon plants of the genus *Jussiæa*, already mentioned. So far I have been unable to find any mention of the root system of *Mikania* by any observer; neither have I talked with any botanist who has noticed them. At the Rochester (1892) meeting of the American Association for the Advancement of Science I presented a preliminary note upon the subject, expecting that if any of the members of the section of biology had noticed them they would make it known. Prof. W. P. Wilson (37 and 38), of the University of Pennsylvania, who has carried on continued and exhaustive investigations upon cypress knees and other aërating structures, told me at that time that he had never noticed any peculiarities in this plant, but had never made that plant a subject of special study. I have had no opportunity to travel and examine the plant in a wide range of localities. Of course the root system of such a plant would never be found upon an herbarium sheet.

Description of the Root System of Mikania.[*]

Many aquatic or semi-aquatic plants develop roots that exist in the water and never reach the soil. *Mikania* produces a great number of such roots. The plant is perennial; its stem dies each autumn to or below the surface of the water. From this stump new shoots arise the following spring. In this respect the plant behaves much as does our other perennial compositæ. Before the young shoots appear in the spring, however, a few long floating roots are sent out from the half-dead stump. The plant shows little activity during the early part of the season. The roots do not increase very fast, and except for the presence of the multitude of now half-decayed roots of the previous season the plant would appear in all respects like any other swamp plant. The young shoots as they come toward the surface of the water have a yellowish-green color, which changes to bright green as soon as they emerge from the water.

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[*] In speaking of *Mikania*, *M. scandens* is always meant.

As the plants grow, the number of roots increase and the main roots begin to send out numberless thread-like branches. The branching of the root system increases until after the plant has frozen down in autumn. The root system remains alive until the water freezes over. I have even taken roots of plants up through ice one foot thick and found many of them perfectly alive. Only a few live until spring, and those are soon replaced by the new roots of the spring.

Two kinds of aquatic roots appear: First, those which are wholly submerged, or in low water merely float upon the surface. (See Plates I and II.) In these the plant seems not only to send its roots up to the air, but also to try to expose as much surface as possible to the surrounding water. The drawing shows only a small portion of one root. It will be noticed that although these roots are very flexuous, the most of them arise from the upper side of the main root and, as they float, take an erect position. These roots are very thread-like and much smaller in diameter than the main roots. The roots which take on the second form occur upon the plants which are in the mud rather than in the water. (See Plates I and II.) The branches of the main roots are fewer, shorter, and thicker than the thread-like roots of the first class. They are more inclined to be branched. They are aërotropic, however, and their tips are often just above the surface of the water. The drawings do not show what an immense root surface is exposed in these plants.

Effect of Transplanting.

Before proceeding to describe the minute structure of these roots it will be well, perhaps, to note the results of an experiment in transplanting these plants to a dry place.

The roots were first noticed by the writer when collecting fruits of the plants along the banks of the Oswego river, in New York State, in the autumn of 1888. A few plants were transplanted to the garden of the University. They were put in a place where the ground would be tilled occasionally. The rather dry surroundings did not seem to be favorable to their growth, but they have lived until the present time (1893), and have sometimes made feeble attempts to flower. The roots still persist in sending up every autumn the characteristic "knees." These arise above the surface of the ground and soon become conspicuously purple, although in the water they were white. A mass of earth and roots was taken out of the ground and potted. It occurred to me that it would be interesting to see what the effect would be if this mass of roots was submerged. The roots had ceased to grow when they were taken out of the ground, and it seemed to me extremely doubtful whether or not they would begin growth again. They had been submerged but a few days when the roots began growth again and continued until they reached the surface of the water.

To conclude the remarks upon the root system, we may say that:

(1.) The root system of *Mikania scandens* exposes a much greater superficial surface than do aquatic plants generally.

(2.) This root system is, for the most part, an annual growth.

(3.) It begins growth earlier in the spring and ceases growth later in the autumn than does the growth of the parts above the surface of the water.

(4.) The branches of the main root tend to be aërotropic, whether they are in shallow water or mud or deeply submerged.

(5.) So strongly is this peculiarity fixed upon the plant that when it is transplanted to dry soil the aërotropic roots still continue to develop.

(6.) Experiments show that the aërotropic roots will begin growth again when placed in water, no matter whether they began growth in the soil or in water.

The Minute Anatomy of the Roots.

For convenience the aërotropic branches of the root system will be called rootlets; the main branches, roots.

Rootlets.

These are characterized by the great development of intercellular spaces in the cortex and in the phloem portion of the radial bundle. There are four phloem, four xylem masses in the central cylinder. Very near the growing point the cells of the cortex begin to round off, leaving between them a four-angled space. A little farther from the growing point the cells spread still farther apart, and there is brought about the arrangement of cells characteristic of these roots. It is different in origin from the tissue which Schenk (35) called aërenchyma. This is from the growing point, and hence is a primary tissue. That which he described is a secondary tissue coming from a periderm. The stages through which the cells pass is strikingly similar in both cases. The great elongation of cell-arms ("Zellbalken") is wanting in *Mikania*. What Schenk (35) paid too little attention to in his very valuable article was the relation of the protoplasm containing cells to the air spaces. Indeed, it seems to me that the same criticism may be extended to most structural studies. The framework of the plant is minutely described, but the presence and nature of the cell contents are entirely neglected.

The nuclei of the cells in the cortex of the rootlets are large. There is also a well-marked ectoplasm lining the wall of each. The epidermal layer in the younger portion of the root is made up of cells rich in protoplasm. Outside of this, toward the growing point, are the cells of the root cap. The epidermis proper has a layer of small cells outside of it. The intercellular spaces begin to appear immediately within the epidermis. The epidermis is delicate, and only slightly cutinized. Toward the inner part of the cortex the cells remain in concentric layers. The xylem is only feebly developed. Sometimes it seems to be reduced to *nil*, in other cases a narrow vessel or two can be distinguished. These have spiral markings. The phloem is much more clearly marked. The richness of its cells in protoplasm is well brought out when sections are stained with hæmatoxylin. A distinct endodermis surrounds the plerome and its cells are in contact with the outermost cells of the phloem. Four cells, two belonging to the endodermis and two to the layer of cells just outside it, are peculiarly modified. The cells are so arranged as to form the walls of an intercellular space which is a continuous tube through the phloem. Sometimes more than one tube is formed. When this is the case they may be arranged radially or concentrically. I have never found them and I feel sure that they do not often occur anywhere in the plerome except at the phloem portions of the bundle. The central portion of the plerome is made up of cylindric cells with a length perhaps twice their diameter. I wish to record here the observation that the nuclei of the cells in the phloem which line the air spaces uniformly lie against the side of the cell which is in contact with the intercellular space. The significance of this relationship between the nuclei of the cells and the air spaces is difficult to understand upon any other supposition than that they find more ready diffusion of air at that point than they would upon the remote side of the cell. If we may consider each cell as an individual, they might be compared to certain organisms which find the most congenial conditions at the surface of the water. The air in the intercellular spaces maintains its purity through the diffusion of gases through the cell-walls. This has been conclusively shown by Devaux (39). Farther, the air diffused through the cell-wall is destined, in parts not green, to be deprived of its oxygen by the protoplasm in the process of respiration. Protoplasm, to live, must be provided with oxygen. The intercellular spaces provide ready means for the circulation of gases, and in a certain sense regulate the proportion of gases mixed in them. On that account some of the eminent French investigators have called intercellular spaces "regulators of gases."

Before going on with a study of the structure of the main roots it will be interesting to glance for a moment at the internal structure of the rootlets of the plants in the garden. Their general behavior, as we have already pointed out, is much the same whether in the mud or transplanted to a dry place. I have observed two peculiarities of structure in the roots in water—large spaces in the cortex and smaller ones in the phloem of the central cylinder. The latter persists as a characteristic of the rootlet even after the plant has been cultivated three years. There is seldom, if ever, more than one of these spaces in the phloem of the dry-land plant, whereas in the submerged rootlets there are often two or three. The xylem is more evident, too, in the specimens transplanted to the dry land. This is in accord with M. Constantin's (23) observations. The large spaces of the cortex are not so highly developed in the dry-land rootlets. Spaces, of course, exist, as indeed they do in nearly, if not all, plant tissue, but they are much smaller.

The Main Roots.

Mention has already been made of these roots. They are the first to appear in the spring. Their capacity for giving off lateral branches is early shown, but only reaches its greatest manifestation in the summer and autumn. They spring in great numbers from the nodes of the stem. The origin of tissues in these roots is the same as in the rootlets. The cortex contains large intercellular spaces. These are surrounded by cells which do not have as prominent protoplasmic contents as do the cells lining the spaces of the rootlets. The intercellular spaces in the phloem are either wanting or inconspicuous. The xylem is more strongly developed.

The Formation of Spaces in Roots of Mikania.

In the delicate organs here under consideration there is an excellent opportunity to study the separation of cells to form intercellular spaces. De Bary distinguishes two kinds of intercellular spaces—(1) schizogenetic and (2) lysigenetic. The spaces in the cortex of the root are of the former type. The unusual case described further on (see Plate IV), where almost the entire central cylinder is wanting, is of the second type.

Schizogenetic Origin of Spaces.

A cross-section of a root tip of *Mikania* shows the cortex to be made up of regular concentric layers of cells. So regular in thickness are the layers that one might easily lay out a plan of it with a pair of dividers. A few of the outer layers are less regular than the inner ones, however. The increase in area toward the circumference is formed without extensive enlargement of the cells by one cell being replaced by two in a radial row. The first appearance of spaces in the cortex is brought about by the splitting of the cells at the angles. In the early stages these spaces are very regular and universally present between the cells. Thus the spaces, as well as the cells, form radial rows from this aspect. Without knowing more of their structure than can be seen in cross-section, one might take them for a bundle of glass tubes set up in a regular manner and then a cross-section of them taken. An examination of the longitudinal section of the tissue shows that the spaces are not uniform throughout, and that from the first there is a tendency to form compartments. From this aspect the cells are fusiform and placed end to end. As the tissues become older from some influence or other—probably the unequal pressure of gases—the spaces become more conspicuous, until they constitute at least one-half of the mass of the tissue. This may very properly be called "spongy tissue." The occurrence of minute openings between each compartment in the "spongy tissue," which is so evident in *Menyanthes*, is much less conspicuous in these roots. They are present, however, and enable the gases to pass from space to space by effusion. The only means of entrance to the spaces is by diffusion through the epidermal cells. These are but slightly cutinized and are very completely filled with protoplasm. That this protoplasm assists in the act of diffusion seems to me highly probable. The difficulties attending the direct demonstration of the function of this living membrane are so great that no one claims as yet to have actually shown it to assist actively in the process. The spaces within the periderm, already alluded to, originate, as do those of the cortex, at the angles of the cells. They do not, however, pass over into compartments, as do the spaces of the cortex, but remain as tubes parallel to and in contact with the cells of the phloem of the root and extend down nearly to the growing point.

I am not aware of record having been made of these openings. Recently in the embryo of certain species of the same order similar spaces were observed, and no doubt many other young organs may have them. Indeed it seems not improbable that these spaces are to the phloem what trachea of the xylem are to the phloem of the bundles in more mature organs.

Bearing of Investigations upon the Office of the Vessels of the Fibro-vascular Bundles.

The office of the vessels of a fibro-vascular bundle has never been satisfactorily explained. At one time it was believed that it conducted the juices of the plant from one organ to another, but it was found that they usually contained air. The passage of water has been found to be mostly by inhibition in the cell-wall or in the protoplasm of the cells. There can be no doubt of their efficiency as ventilating structures. The cells of the phloem are the ones which are most active functionally, and consequently require aëration. If in young organs conspicuous air spaces are provided to accompany them, it is not difficult to conceive of vessels of the xylem as performing the same function in older organs. I do not mean to say that we have proven what the office of the vessels of the tracheary tissue of plants is, but I do wish to say that there are structural evidences pointing toward the foregoing explanation of their use.

A demonstration of the presence of the intercellular air-spaces in the young roots can easily be made by treating one of them with sulphuric acid. After treatment the tissues become transparent and the air becomes visible as black lines extending down nearly to the growing point and often emitting bubbles from the cut end. These black lines are the larger intercellular spaces, and their form indicates how they serve both as receptacles for and distributors of gases. I have treated young roots in this way frequently and have found air present in their spaces. The same treatment shows that the epidermis of the root is only slightly cutinized.

Summary of Remarks Relating to the Minute Anatomy of Mikania Roots.

1. The internal structure of the roots is characteristic of the structure of the submerged organs of aquatic plants as made out by M. Constantin (23).
2. The reduction of the xylem and the increase in capacity of the intercellular spaces are conspicuous features in the submerged organs.
3. When grown in the garden for a period of years the organs tend to develop a more perfect xylem and at the same time reduce the size of intercellular spaces of the cortex.
4. Gases may pass from one compartment to another by effusion as well as diffusion. The openings between spaces are not as conspicuous as they are in some other species; for example, in Menyanthes.
5. Gases to pass into the organ must traverse a living continuous membrane. This they do by diffusion.
6. Intercellular spaces occur in the phloem of the radial bundle. These spaces have large cells lining them, the nuclei of which have an affinity for the side of the cell which borders the air space. This is presumably because the conditions of life are better at this point.

The Physiological Significance of These Organs.

Mikania has two features which lead me to believe that it adapts its organs to secure aëration—1st, the aërotropic tendency of its roots; 2d, the intercellular spaces in the same organs.

I can cite no stronger evidence of the use of these modifications than has been set forth in the discussion of their structural details. The same conclusions have been drawn by others who have studied aërating structures. Wilson (37 and 38) and Shaler (30 and 31) have assigned that function to "cypress-knees," as did also Dickeson and Brown (5), the first men to investigate them. Martins (10) and Schenk (35) have studied the aërotropic roots of the South American *Jussiæas.* Jost (29) has studied the influence of too much moisture upon the roots of palms and other related plants and found that excessive moisture led to decided aërotropic tendencies in their roots. He insisted that it was not negative geotropism but aërotropism that occurred.

It was but a few years ago that aërenchyma tissue was first described. It is of comparatively little importance whether that tissue, as described by Schenk, should be given a distinctive name or simply looked upon as modified parenchyma. It is my opinion that the latter would be the wiser course, for if we attempt to classify tissues with reference to their functions we soon get into hopeless confusion ; nor is it to be distinguished from other tissues by its origin. It has the same origin as cork and, indeed, all other tissues arising from secondary thickenings outside the cambium.

The great variability in the proportion of oxygen and carbon dioxide in the gases contained in the intercellular spaces has been demonstrated over and over again. It has also been shown that in chlorophylless organs the proportion of oxygen in the gases of the air spaces tends toward having the same proportion of oxygen as is contained in the free atmosphere. It never attains to that amount, however. Martin's analyses of the gases contained in the spaces gave these results, as did the investigations of Bonnier (24, etc.), Mangin (24, etc.), and Devaux (39). It is, then, proper that the last-named investigator should have applied the term "regulators" to the intercellular spaces of plant tissues.

Constantin (23) and Lewakoffski (18) have experimented upon plants with reference to their adaptability to aquatic or terrestrial conditions. The former planted terrestrial plants in water and also aquatic plants upon dry land, the results of which I have already mentioned. If it should be asked why there should be such a correlation between structure and environment, I can simply say that aquatic organs would never withstand the hardships of a terrestrial environment, and that the reason for a strong tracheary system not being developed is simply because it is not needed.

Methods.

The methods of investigation pursued upon these roots must be stated before the work can lay any claim to completion. I thoroughly believe that had I depended upon dissections and sections cut from fresh material no correct idea of the intercellular system of the plant could have been possible. The organs investigated are so delicate that the mere handling displaces and crushes their tissues.

A method of fixing is absolutely necessary to a correct understanding of them.

The improvement in histological methods was undertaken in order to make possible the investigation (in place) of the most delicate organs. Prof. M. B. Thomas, of Wabash College, at the time the investigations were begun (1890), a student and later a fellow in Cornell University, took up the collodion method of imbedding vegetable tissues, and the paraffin method, which had already been used to some extent in making sections of vegetable tissues, was taken up by myself. The work upon methods was suggested and constantly encouraged by Professor Gage, who was at that time using them with good results in animal histology. The results of Professor Thomas' investigations were published in the Proceedings of the American Microscopical Society for 1890. My own investigations upon the paraffin method were published in the same place. We tested both methods carefully and found that the collodion method was simpler and gave the best results. Since that time collodion has been used in the laboratory with uniformly good results.

The tissues to be cut are treated in the following manner: The objects to be cut are trimmed as desired, then placed in weak alcohol (20 to 40 per cent.), depending upon the delicacy and succulency of the tissue. The strength is gradually increased. This is done most satisfactorily by a Schultze's dehydrating apparatus. The principle of this apparatus is the gradual diffusion of liquids of different densities through a membrane. A membrane of chamois-skin serves the purpose very well. By this treatment the tissues are "fixed" or hardened. After hardening, the object, if delicate, must be handled with even more care than before it was fixed, as the tissues are no longer flexible. After hardening in the dehydrating apparatus until the objects are and have remained for an hour or more in 95 per cent. alcohol they are transferred to 2 per cent. collodion, which is made by dissolving two grams of soluble cotton* in 100 cc. of alcohol mixed with an equal quantity of sulphuric ether. Care must be taken to have no water in the solution, as even a small amount will spoil the collodion. After the object has remained in the 2 per cent. collodion for from 4 to 10 hours, depending upon the size and density of the object, it is transferred to

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*We use soluble cotton manufactured by Charles Cooper & Co., 194 Worth street, New York.

5 per cent. collodion, made exactly like the thinner solution, except that five grams of cotton are used instead of two. In this thick collodion the objects remain as long as they did in the thin solution.

After considerable experience we have found that the less delicate objects are handled the better. Instead of mounting, as has often been recommended, directly upon cork, we pour the collodion, object and all, out into a paper tray. The collodion is permitted to harden in the air until it is sufficiently stiff not to flow; then it is put in alcohol or chloroform to harden. After three or four hours the collodion can be cut up in blocks and either cut by fastening the blocks direct in the microtome or they may be cemented to a cork with fresh collodion, which must be again hardened. When sections of objects are fixed, infiltrated, imbedded, and cut according to these directions it will be found that the protoplasm remains fixed and the walls of the cells are not displaced. The collodion which is infiltrated into the tissue may remain without in any way interfering with the observation of the tissues. In this respect collodion is much better than paraffin.

I have stained my sections almost exclusively with hæmatoxylin. The sections are mounted in balsam.

Success in photomicrography necessarily depends to a large extent upon the perfection of the objects to be photographed. A poor mount will invariable give a poor photograph.

The plates which accompany this paper were traced from photographs of the objects themselves.

It is perhaps superfluous, after what I have said about the adaptations of structure in *Mikania*, to add that I believe it is very important that investigations upon the minute structure of organs should be carried on, and that upon those investigations must be based largely the interpretations of the uses of organs and tissues.

(The investigations recorded in this thesis were carried on in the botanical laboratory of Cornell University.)

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Description of Plates.

PLATE I.

FIG. 1.—Mass of roots borne by submerged portion of stem of Mikania. When in the water the thread-like branches of the root system reached the surface of the water. Notice the tendency to produce branches from the upper side of the main root. × $\frac{1}{10}$.

FIG. 2.—Root system of plant grown in dry garden. The branches are still aërotropic, but are much reduced in number. × $\frac{1}{5}$.

(Both drawn from photographs of specimens after removal from water or soil, as were the figures shown in Plates II and III)

PLATE II.

FIG. 1.—Mass of roots of *Mikania* taken from shallow water. A small portion of this mass of roots is shown in Fig. 1, Plate III. × $\frac{1}{10}$.

PLATE III.

FIG. 1.—Portion of root system of a plant growing in shallow water; but a single node of the submerged stem shown, from which several main roots grow. The latter bear the aërotropic roots. × $\frac{1}{5}$.

FIG. 2.—Three nodes of a deeply submerged stem similar to Fig. 1, Plate I. Note the comparative abundance and length of the aërotropic roots. In this specimen four nodes of the stem are represented. The three upper bear young roots. × $\frac{1}{5}$.

PLATE IV.

FIGS. 1 and 2.—Cross and longitudinal sections of young aërotropic root near the root-tip. Camera lucida drawing designed especially to show the relation of the cells and their contents to the intercellular spaces: i. = intercellular spaces of the cortex; c. = cortex; ph. = phloem; rc. = root-cap; ep. = epidermis; en. = endodermis; pl. = plerome. × $\frac{425}{1}$.

PLATE V.

FIGS. 1 and 2.—Cross and longitudinal sections of large hollow root of Mikania: xy. = xylem; otherwise letter as Plate IV. × $\frac{250}{1}$.

PLATE VI.

FIG. 1.—Cross section of portion of root in the region of phlœm. Camera lucida drawing showing the intercellular spaces, the relation of the cells to them, and especially the position of the nucleii of the cells. Lettered as before. × $\frac{800}{1}$.

FIG. 2.—Cross-section through the growing point of a rapidly-growing main root showing the first indication of intercellular spaces at the angles of the cells. These spaces soon take on the form found in the cortex of the older root, shown in Plate V. × $\frac{200}{1}$.

PLATE I.

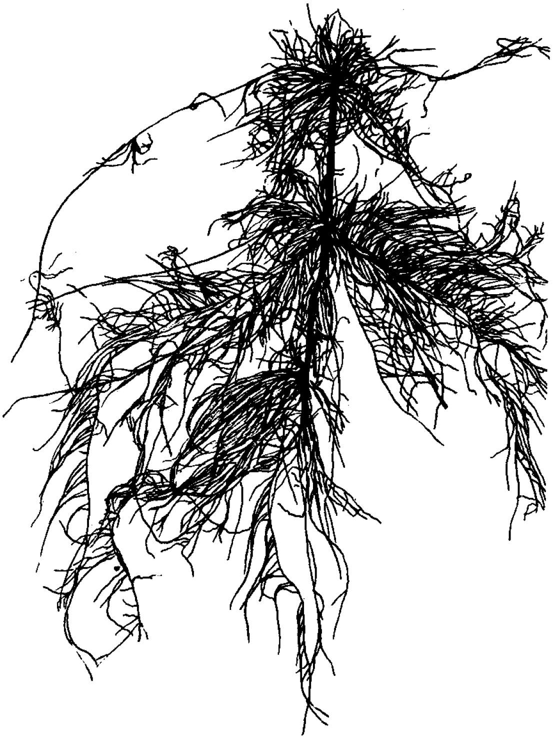


FIG. 1.

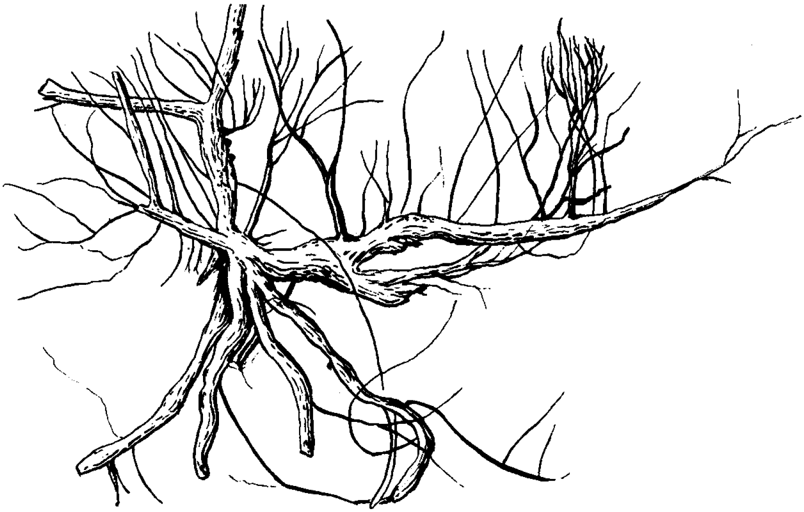


FIG. 2.

PLATE II.

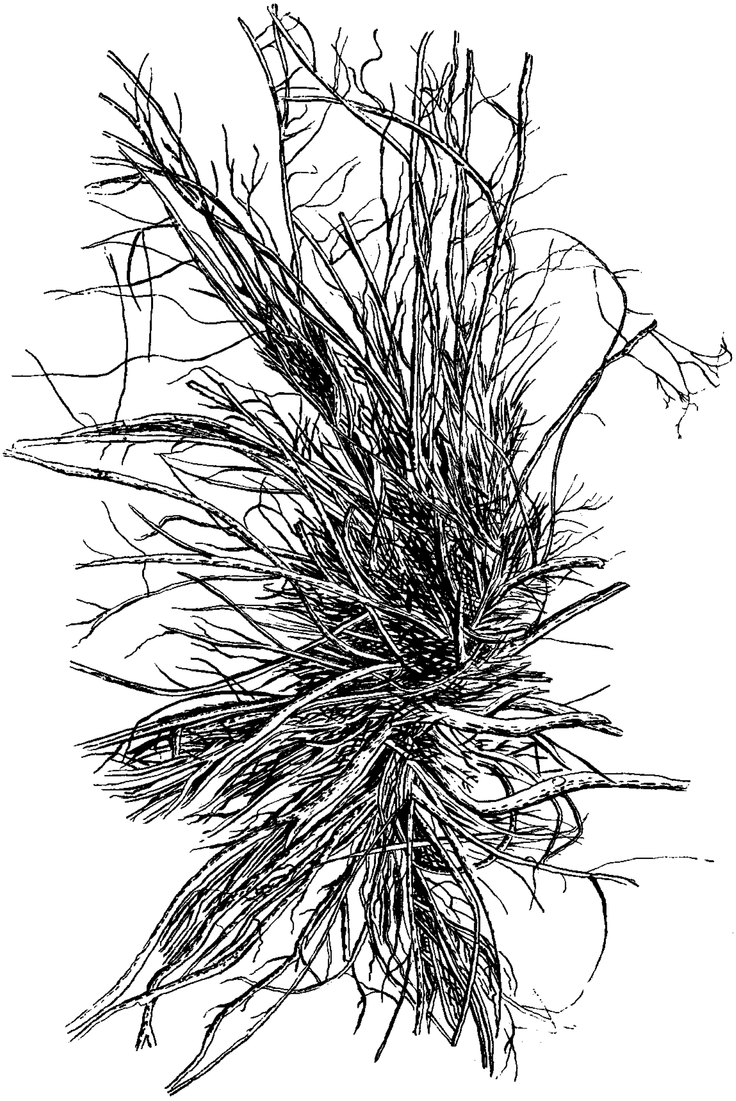
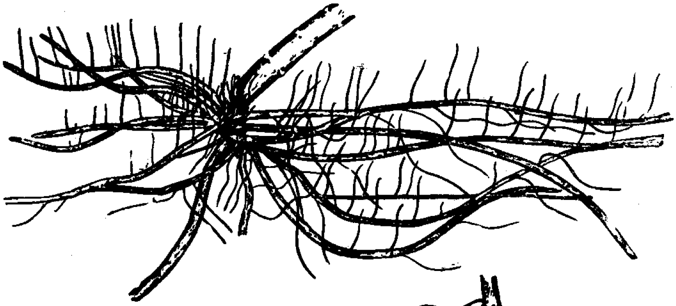


PLATE III.



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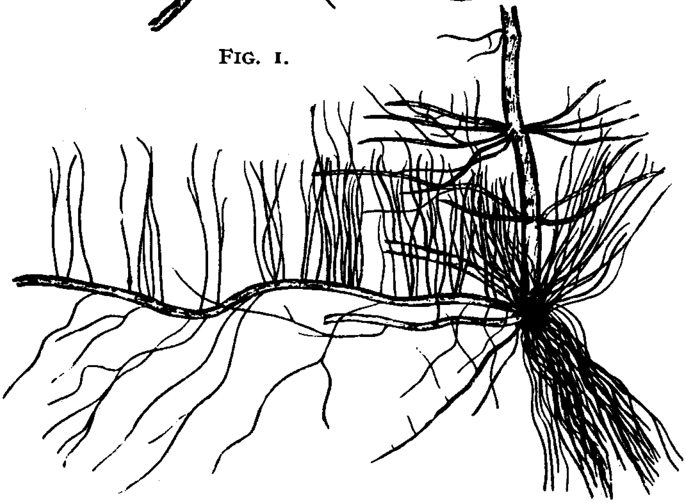
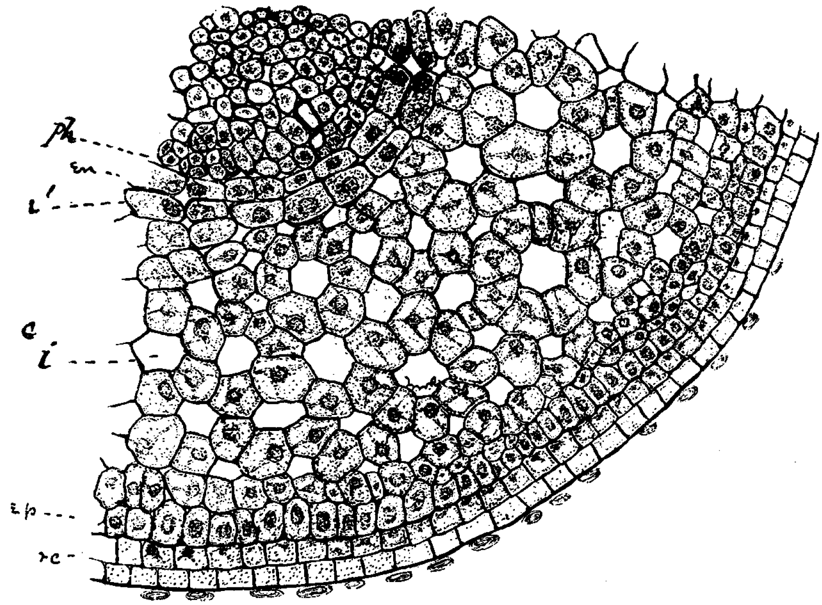


FIG. 2.

PLATE IV.



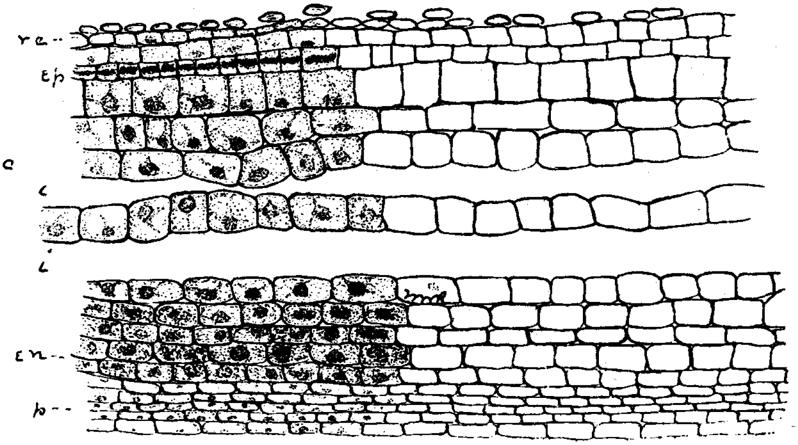
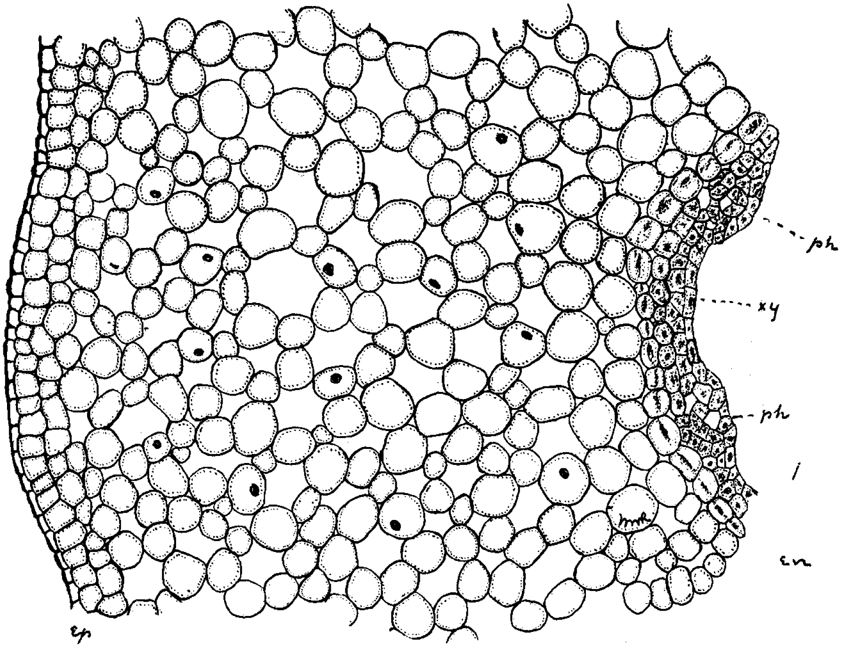


PLATE V.



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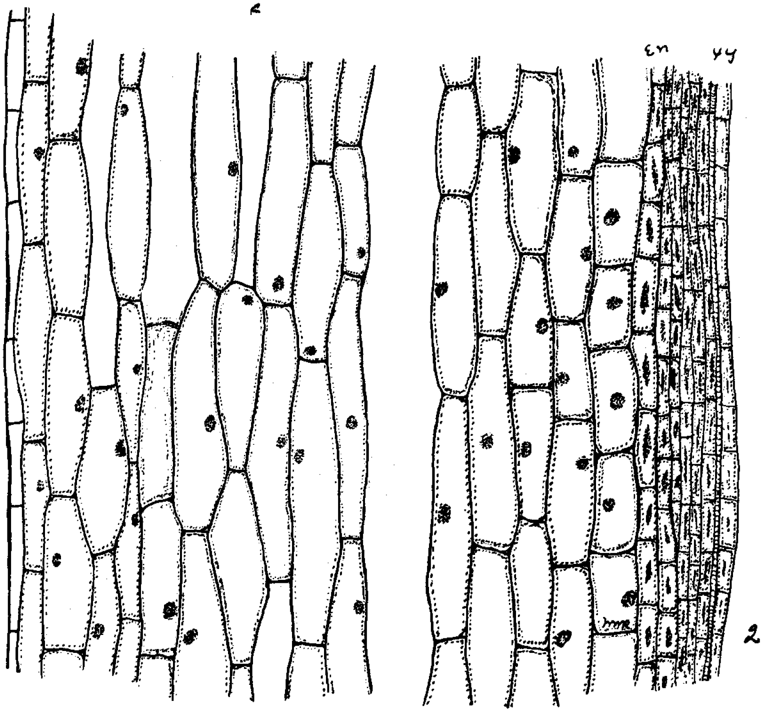

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PLATE VI.